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SELECTED TRANSLATIONS ON IRRADIATION IN MEDICINE AND CHEMISTRY

-USSR-

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SELECTED TRANSLATIONS ON IRRADIATION IN MEDICINE AND CHEMISTRY

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[Following are translations of selected articles on irradiation in medicine and chemistry published in the periodical Voprosy meditsinskoy khimii (Porblems of Medical Chemistry), Vol 6, No 5, Moscow, September-October 1960. Other bibliographic information accompanies the individual articles.]

TABLE OF CONTENTS

THE EFFECT OF EXTERNAL IONIZING RADIATION ON THE PARTICIPATION OF THE LIVER AND INTESTINE ON LIPID METABOLISM

-USSR-

[Following is the translation of an article entitled "Vliyaniye vnesheego ioniziruyushchego izlucheniya na uchastiye pecheni i kishechnika v lipidnom obmene" (English version above) by K. V. Smirnov and V. A. Shaternikov in Voprosy Meditsinskoy Khimi (Problems of Medical Chemistry), Vol 6, No 5, Moscow, Sept-Oct 1960, pages 464-468.]

Lipid metabolism disorders command considerable interest in studying the pathogenesis of radiation sickness. Ionizing radiation inhibits the process of lipid absorption from the gastro-intestinal tract, and increases lipid excretion in the feces. The increase in the blood concentration of low density lipoproteins, testifies to the fact, that assimilation of exogenous lipids by the organs and tissues, is also made more difficult. On the other hand, a number of authors have shown, that following radiation, fatty infiltration of the liver is observed. It was further established, that there is an intensified synthesis by the liver and certain other organs, of lipid components, including neutral fats, phospholipids and cholesterol. In the early stages following radiation, there is an increase in the blood content of all lipids, including neutral fats and cholesterol.

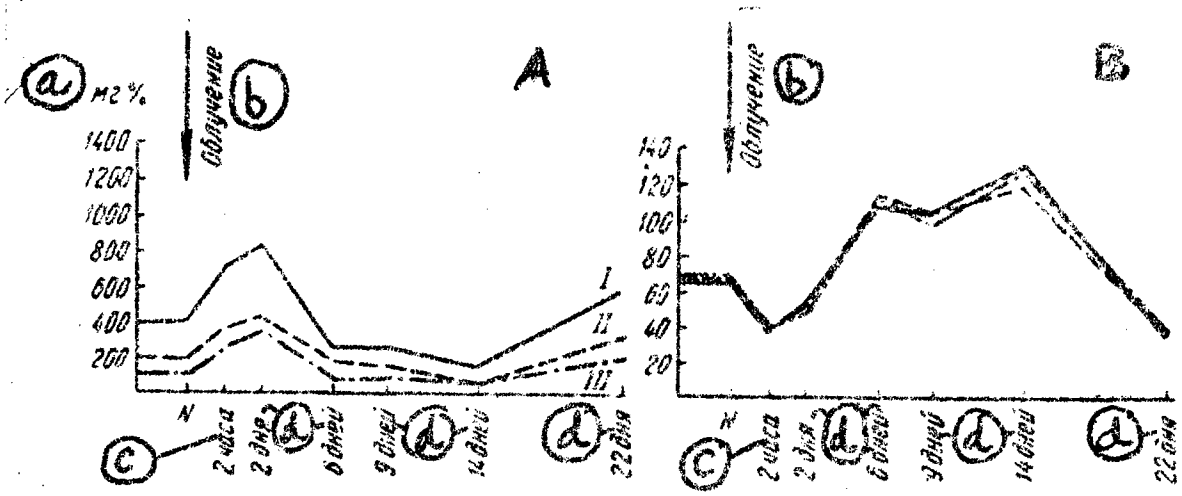
The present work attempts to discover the role of the liver and intestine in the dynamics of lipid exchange and blood fractionization, following stimulation by ionizing radiation.

Research Methods and Results

Experiments were conducted on five dogs, angiostomized by the Lonion method, with our modifications (isolated strips of small intestine were used as cannulae). We examined blood taken from the femoral artery, the portal and hepatic veins. In the collected samples, we determined the general fat content, the amount of neutral fats and esterified cholesterol (henceforth

referred to as the amount of neutral lipids), phospholipids, by the Blur method, as well as the iodine value of these fractions, using the Rosenmund-Kunchenn method. The examined blood was taken from animals on an empty stomach, 18 hours after feeding.

After a standard physical examination, the animals were subjected to a general single γ-irradiation with Co^{60} at a dose of 300 r and intensity of 450-460 r/min. Post-radiation blood analyses were made after two hours, 2, 6, 9, 14, and 22 days.

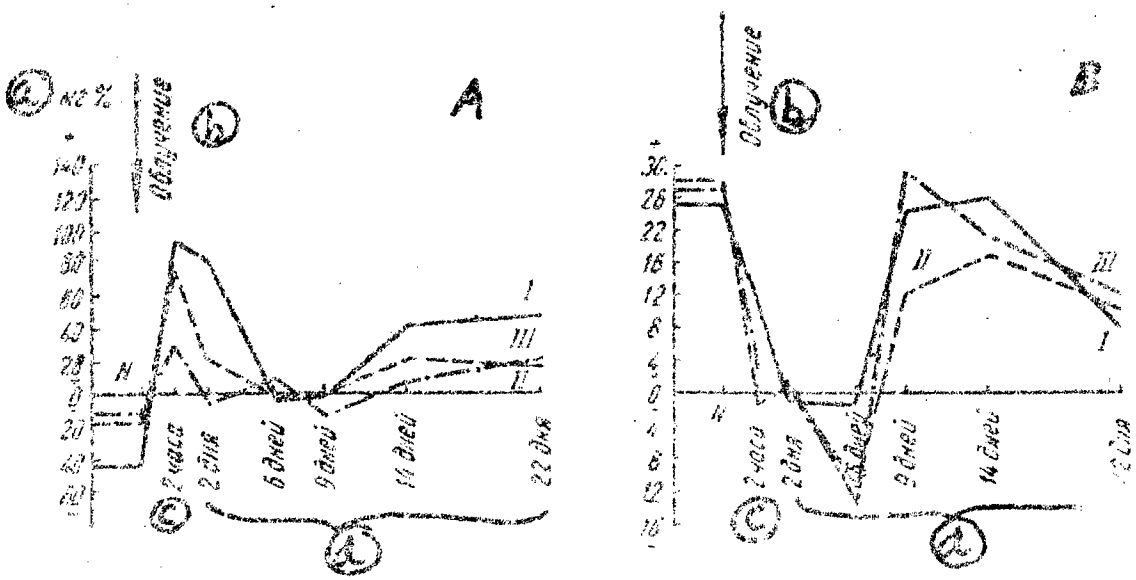


Diag. 1. Content (A) and iodine value (B) of lipids and their fractions in the arterial blood of dogs after a γ-irradiation dose of 300 r.
I— general lipids; II — neutral fats and esterified cholesterol; III — phospholipids.
Legend:
a) mg %
b) irradiation
c) hours
d) days

The results of our determinations indicated (diag. 1), that under our conditions, the general lipid content of arterial blood plasma in healthy dogs on an empty stomach, averaged 410 mg % (from 333 to 514 mg %). Of this amount, neutral lipids constituted 197 mg % (from 152 to 250 mg %), phospholipids — 118 mg % (from 56 to 174 mg %). The iodine value of general lipids was equivalent to an average of 66.9 (from 51.2 to 83.9), neutral lipids — 68.5 (from 58.9 to 74.5), phospholipids — 67.2 (from 54.2 to 77.6).

Two hours after irradiation, the content of general lipids, neutral lipids and phospholipids sharply increased in the arterial blood plasma, whereas the iodine value decreased (see diag. 1).

Two days after irradiation, the blood lipid and lipid fraction content increased further, whereas the iodine value did not significantly change . The sixth day was characterized by a decrease in the content of general lipids, neutral lipids and phospholipids lower than normal, and by an increase of their iodine value significantly higher than the original level.



Diag. 2. Content changes (A) and iodine value changes (B) of lipids and their fractions in dog blood during its passage through the intestine.
Symbol designations are the same as in diag. 1.

In subsequent periods (9th and 14th day), the content of general lipids, neutral lipids and phospholipids in the arterial blood remained low, but their iodine value was high, as before. In the terminal period of radiation sickness (times varied in different dogs), a hyperlipemia was again observed, that is, an increase in the concentration of general lipids, neutral lipids and phospholipids higher than the original level, and a decrease in the iodine value below normal.

The role of the intestine in the metabolism of lipids in radiation sickness is indicated by data represented in diagram 2.

The concentration of general lipids, neutral lipids and phospholipids (retained by the intestine) in blood passing through the intestinal wall of healthy animals on an empty stomach, decreased, whereas their iodine value increased, as did their degree of unsaturation.

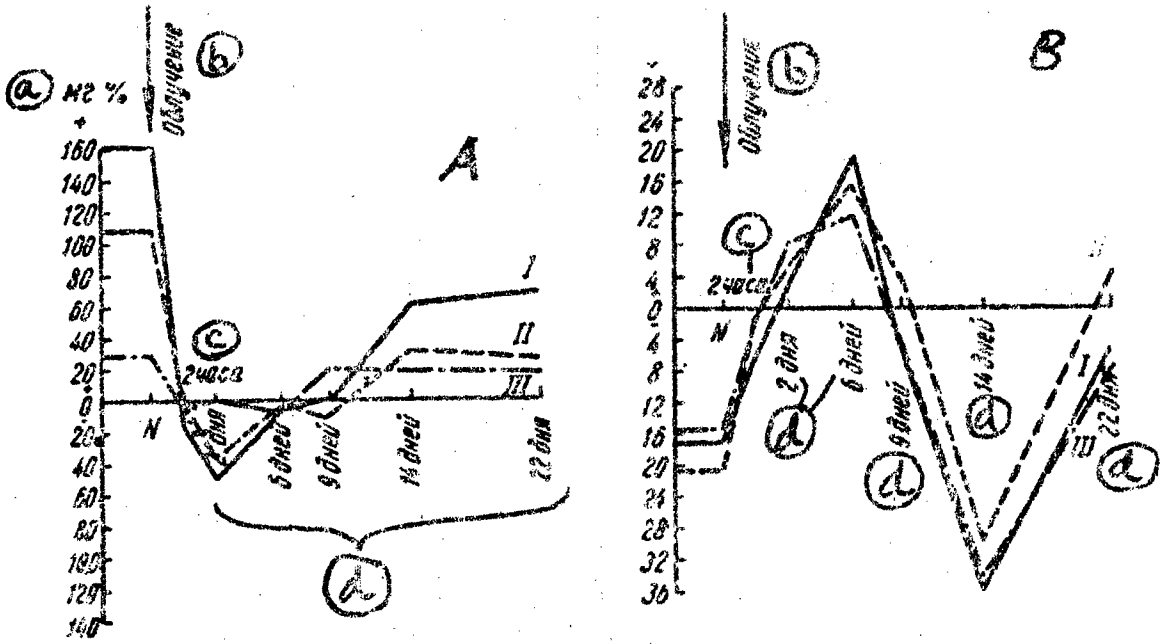
Two hours after irradiation, when a general increase in the lipid arterial blood level was observed, the intestine further increased the blood concentration of general lipids as well as

neutral lipids and phospholipids. The iodine value of general lipids and phospholipids in blood passing through the intestine increased significantly less than before irradiation, whereas the iodine value of neutral lipids actually decreased.

Two days after irradiation, during a still further increase of the arterial blood lipid level, the intestine continued to return general lipids, and in particular, neutral lipids, to the blood, although in a somewhat lesser quantity; the intestine again retained phospholipids, but to a lesser degree than before irradiation. During this period, the lipid iodine value in blood passing through the instestinal wall did not significantly change.

Six days after irradiation, when the concentration of lipids in the arterial blood was significantly below normal, the intestine retained a certain quantity of general lipids and neutral lipids, and returned phospholipids to the blood. Blood lipids passing through the intestine became more saturated at this time, especially the neutral lipids and phospholipids.

The ninth day was characterized by the absence of any influence of the intestine on the level of general lipids and neutral lipids in the blood plasma, and by some retention of phospholipids. The intestine, however, sharply increased the degree of lipid unsaturation.



Diag. 3. Changes in the content (A) and iodine value (B) of lipids and their fractions in dog blood during passage through the liver.

Symbol designations are the same as in diagram 1.

Fourteen days after irradiation, as the lipid level in the arterial blood continued to be low, the intestine returned general lipids, neutral lipids and phospholipids to the blood, and increased the degree of unsaturation of lipid fractions.

The role of the liver in lipid metabolism in acute radiation sickness is characterized by the data represented in diagram 3.

The liver of healthy, non-irradiated dogs on an empty stomach, excreted, into blood circulating through it, general lipids, neutral lipids and phospholipids, increasing their concentration. Consequently, the iodine value of the lipid fractions decreased. In this manner, the effect of the liver, under normal circumstances, on blood lipids is opposite to that of the intestine as described above.

Two hours after irradiation, the quantity of general and neutral lipids excreted by the liver into the blood significantly decreased, whereas the liver actually retained phospholipids. This was occurring at the same time as the intestine was returning general, neutral and phospho- lipids to the blood. Parallel to this, there was a below-normal significant decrease in the iodine value of lipid fractions.

After two days, as the intestine continued to transfer general and neutral lipids to the blood, but in lesser quantities, and was retaining phospholipids, the liver was depleting the blood of general and neutral lipids and did not show any noticeable effect on the blood content of phospholipids. Concomitantly, the iodine value of the lipid fractions in the blood passing throught the liver, increased.

On the sixth 24 hour period after radiation, as the intestine was retaining a certain amount of general and neutral lipids of the blood, and transfering phospholipids to the blood, the liver was transfering general and neutral lipids to the blood, while retaining phospholipids. As a result, the liver further increased the degree of unsaturation of lipid fractions.

Nine days after irradiation, general and phospholipids were being mobilized from the liver to the blood, although neutral lipids continued to be retained by the liver. At this period, phospholipids were being mobilized from the intestine to the blood, and no noticeable effect by the intestine on general and neutral lipids was demonstrated. At this time the liver did not significantly change the degree of lipid fraction unsaturation.

On the 14th day after irradiation, as general, neutral and phospho- lipids were being mobilized from the intestine to the blood, the liver also increased the lipid fraction concentration in the blood passing through it. Subsequently, the degree of lipid fraction unsaturation decreased.

The data recorded by us in examining the arterial blood, are generally in accord with that of the literature — after irradiation at a dose of 300 r, the general lipid content begins to

increase two hours after radiation stimulation; this hyperlipemia reaches a maximum on approximately the second day of the radiation sickness development, and on the sixth day it is possible to ascertain the lipid content decrease. It subsequently remains low, and a repeated hyperlipemia again develops in the terminal period. In this connection, the parallelism in the content changes of neutral lipids and phospholipids warrants attention. A significant increase in the content of unsaturated fatty acids in the arterial blood lipids takes place at the peak of radiation sickness.

The results of our experiments shed some light on the role of the intestine and liver in lipid metabolism disorders following stimulation by ionizing radiation. It may be assumed that the intestine is largely responsible for the ensuing changes in the blood lipid structure under these conditions, inasmuch as the increase in the blood lipid fraction concentration coincided with the time of the lipid fraction release by the intestine. This is characteristic for the early stages and the terminal period of acute radiation sickness.

It should be noted, that along with the blood flowing directly from the intestine to the portal vein, is the blood from the omentum and mesentery, i.e. from adipose tissue. It is therefore quite possible, that the hyperlipemia in the beginning and terminal stages of radiation sickness is conditioned by the mobilization of fat from the fat depots. Data on the mobilization of fat from the fat depots in various types of stress may serve as indirect confirmation of this.

As regards the liver, in our opinion, it exhibits a compensatory effect on lipid metabolism disorders of the organism both at the initial and peak stages of acute radiation sickness. However, in the terminal period of acute radiation sickness, there occurs a decompensation of liver function which aggravates the lipid metabolism disorder.

Conclusions

1. Following irradiation of dogs with γ-rays of Co^{60} at a dose of 300 r, development of a hyperlipemia was observed in the early stages and terminal period of radiation sickness.

2. The intestins of animals, in the early stages and terminal period of radiation sickness, supplied a significantly greater amount of lipids to the blood circulating through it, than before irradiation of the animals.

3. In the early stages of radiation sickness the liver exhibited a compensatory effect on lipid changes in the blood, brought about by its passage through the intestine. However, in the terminal period, both the liver and intestine enriched effluent blood with lipids.

BIBLIOGRAPHY

1. Morehouse, M. J. and Searcy, R. L., Radiation Res., 1956, Vol 4, p 175.
2. Morehouse, M. J. and Searcy, R. L., Science, 1956, Vol 123, p 1032.
3. Hayes, T. L. and Hewlitt, J. E., Am. J. Physiol., 1955, Vol 181, p 280
4. Hewlitt, J. E., Hayes, T. L., Golfman, J. W., et al, Am. J. Physiol., 1953, Vol 172, p 579.
5. Hewlitt, J. E. and Hayes, T. L., Am. J. Physiol., 1956, Vol 185, p 257.
6. Goldwater, W. H. and Entenman, C., Fed. Proc., 1954, Vol 13, p 218.
7. Goldwater, W. H. and Entenman, C., Fed. Proc., 1955, Vol 14, p 59.
8. Goldwater, W. H. and Entenman, C., Radiation Res., 1956, Vol 4, p 243.
9. Ivanov, I. I., Balabukha, V. S., Romantsev, Ye. F., et al, Obmen veshchestv pri luchevoy bolezni (Metabolism in Radiation Sickness), Moscow, 1956, p 127.
10. Coniglio, J. G. and Hudson, G. W., Fed. Proc., 1954, Vol 13, p 104
11. Coniglio, J. G., McCormick, D. B., and Hudson, G. W., Fed. Proc. 1955, Vol 14, p 30.
12. Coniglio, J. G., McCormick, D. B., and Hudson G. W., Am. J. Physiol., 1956, Vol 185, p 577.
13. Sherman, F. G. and Almeida, A. B., Fed. Proc., 1955, Vol 14, p 139.
14. Sherman, F. G. and Almeida, A. B., in the book Advances in Radiobiology, Edinburgh-London, 1956, p 49.
15. Gould, R. G., in the book Progress in Radiobiology, Springfield, 1956, p 71.
16. Grodzenskiy, D. E. and Tret'yakova, K. A., Tezisy dokl. 9-go c"yezda Vsesoyuzn. ob-va fiziologov, biokhimikov, farmakologov (Theses Reports of the Ninth Congress of the All-Union Society of Physiologists, Biochemists, and Pharmacologists), Moscow-Minsk, 1959, p 93.
17. DiLuzio, N. R. and Simon, K. A., Radiation Res., 1957, Vol 7, p 79.
18. Zarafonetis, Ch. J. D., et al, Am. J. Med. Sc., 1959, Vol 237, p 418.

OXIDATIVE AND CARBOHYDRATE-PHOSPHATE METABOLISM IN CEREBRAL AND HEPATIC TISSUE UNDER NORMAL CONDITIONS AND AFTER IRRADIATION

-USSR-

/Following is the translation of an article entitled "Okislitel'ny i uglevodo-fosforny obmen v tkani golovnogo mozga i pecheni v norme i pri obluchenii" (English version above) by R. I. Skvortsova in Voprosy Meditsinskoy Khimi (Problems of Medical Chemistry), Vol 6, No 5, Moscow, Sept-Oct 1960, pages 475-479./

At the present time, radiative biochemistry is replete with extensive material which indicates that one of the most vulnerable metabolic links to radioactivity is the process of oxidative phosphorylation. Both in domestic and foreign literature there is a number of works in which authors have studied conjugative oxidative phosphorylation during exposure of the ganism to ionization. These authors concluded that irradiation suppresses inorganic phosphate binding, coupled with substrate oxidation by tissue homogenates (spleen, liver, thyroid), and mitochondria isolated from tissue (spleen).

It was demonstrated, that local irradiation of the cerebellum and cerebral hemispheres sharply inhibited oxidative phosphorylation.

It would be interesting to see, which of the phosphorytive fractions is most deranged by radioactivity.

Research Methods and Results

Work was conducted on guinea pigs, primarily males, weighing from 250— 350 Gms, and maintained on an ordinary laboratory diet. The animals were subjected to irradiation by a RUM-3 apparatus with the following technical specifications: Current — 15 ma (milliamperes), tube voltage — 180 kw, filter — 0.5 mm Cu, dose capacity — 129 r/min.

Total irradiation dose to the test animals was 1000 r, a 4000 r dose to those with an irradiated cerebrum, and a 9000 r dose /See Note/ to those with an irradiated cerebellum. Liver and cerebellum tissue was examined. Animals with local cerebellar irradiation were tested on the second day after irradiation

at the moment of severest cerebellar derangement. Cerebrally, as well as totally irradiated animals, were tested two to five days after irradiation and later. After weighing, liver tissue was homogenized with a four part volume addition of a 0.25 solution of sucrose; cerebellar tissue was homogenized with a four to five part volume addition of a 1.1 — 0.04 % solution of KCl at a pH of 7.4. The homogenized paste was centrifuged, and the deposit was used as a ferment preparation. An incubating mixture was prepared in the following proportions: 0.5 ml 0.1 M solution of $MgCl_2$; 0.5 ml 0.025 M solution of ATP; 7.5 ml of a phosphate buffer at pH of 7.4 (20 ml of a 1/30 M solution of KH_2PO_4; 80 ml of a 1/30 M solution of $NaHPO_4$); 1ml 0.04 M solution of succinate, and 0.5 ml 0.4 M solution of NaF. 1 ml (3 ml) of the salt solution was used for the incubation, to which was correspondingly added 1 ml (3 ml) of homogenate from cerebellar or liver tissue, respectively. During the incubation, glucose was added to the mixture at a computation of 15 — 30 mg per test. Glycogen, computed from 15 — 20 mg per test, was added during incubation of the liver tissue. [The above-mentioned glucose was added to incubating brain tissue] Several tests were incubated with additions of bromacetate and hexokinase.

([Note] Local irradiation of the cerebellum was conducted by the method developed by P. I. Minayev).

Hexokinase was added to intensify the process, and the added $CH_2BrCOOH$ stabilized the process during the formation of the two phosphotrioses. The incubation was conducted in small oxygen-saturated flasks for a period of 25 minutes at 37°[C]. The degree of phosphorylation was determined by the amount of bound inorganic phosphorus.

In the tests without the addition of hexokinase, the mineral phosphate decrease in cerebellar tissue was approximately equivalent to one mg of phosphorus, and in hepatic tissue, equivalent to three milligrams of phosphorus.

Tests conducted in a nitrogen atmosphere did not give similar results. Phosphorylation was shown to hardly occur during incubation of brain tissue homogenates under anaerobic conditions (table 1).

Table 1.

The binding of inorganic phosphorus during incubation of brain and hepatic tissue in oxygen and nitrogen atmospheres (average data for three trials)

Material examined	Binding of inorganic P in mg per test	
	Oxygen	Nitrogen
Cerebellar tissue . . .	1	0,049
Hepatic tissue	2.83	1.98

This gave us the basis for considering that phosphorylation of carbohydrates (glucose) in brain tissue, proceeds only via the oxidative route.

The phosphorylation of glycogen during incubation of hepatic tissue occurs both in a nitrogen and oxygen atmosphere. It is however less intensive in the former case (see table 1).

The basic problem of the present work was to fractionally divide the phosphorus compounds formed in cerebellar and hepatic tissue during incubation in a O2 atmosphere, under normal conditions and after irradiation.

We conducted the fractionization by the Sacks method, which was proposed by him for the determination of phosphorous compounds of the liver, and modified by N. P. Meshkova and N. V. Aleksakhina.

A general barium precipitate of phosphorous compounds was formed and divided into two fractions: phosphorous compounds of which barium and calcium salts are insoluble in water (Ba — Ca insoluble fraction), and phosphorous compounds of which barium and calcium salts are soluble in water, but insoluble in alcohol (Ba — Ca soluble fraction).

The Ba — Ca insoluble fraction was found to contain phosphorus, inorganic phosphorus, adenosine di- and adenosine triphosphate, and phosphoglycerolic acid; the Ba — Ca soluble fraction was found to contain phosphorous, adenylic acid, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, and glyceryl phosphate.

Experimental studies of the phosphorous compounds showed that, either in the absence or presence of monobromoacetic acid in cerebellar tissue under normal conditions, the diminishing amount of inorganic phosphorous was found, primarily in the form of fructose diphosphate.

Smaller amounts of phosphorus end up in difficult-to-hydrolyze esters (phospho-glycerolic acid and glyceryl phosphates), and in glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate. The amount of ATP decreases in the incubation process.

In studying the phosphorous compounds formed during incubation of irradiated cerebellar tissue homogenates, we discovered that on the second day following irradiation, the primary derangements were in the formation of fructose-1,6-diphosphate. The formation of this product is sharply inhibited. The formation of other phosphorous compounds is simultaneously inhibited, but to a much lesser degree. These include: phosphoglycerolic acid, glyceryl phosphate, glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate. No distinct changes in the ATP — ADP — ascorbic acid system were discovered during irradiation of the cerebellum (see diag. 1).

In the presence of bromacetate, the formation of phosphoglycerolic acid and glyceryl phosphate is reduced, and all processes are converted to the formation of fructose diphosphate.

The inhibition of fructose-1,6-diphosphate formation by radioactivity is quite apparent (see diag. 2).

Two days after local irradiation of the cerebellum at a dose of 9000 r, there was noticeable cerebellar swelling at the time of severest cerebellar disturbances. This forced us to make determinations of the dry residue of cerebellar tissue under normal conditions and after irradiation.

Below, we indicate the content of dry residue in percentages of wet weight (table 2).

In a dry state, equivalent to 16.9 %, irradiated cerebellar tissue increased its humidity, in comparison to the normal, by 23%. In a dry state, equivalent to 19.7%, the humidity is increased by 10 % .

All the results of experiments indicated in diagrams 1 and 2 , are calculated on the basis of cerebellar damp weight. In diagrams 1 and 2 it is distinctly clear, that the binding of mineral phosphate is inhibited (in comparison to the control) by 74 to 56 per cent at the time of severest cerebellar derangement and by 49 to 33 per cent at the time of moderate cerebellar derangement. . one takes into account the edema of the cerebellar tissue, then the inhibition of oxidative phosphorylation during severe cerebellar derangement, will be approximately equivalent to 51 to 33 per cent, and in moderate derangement, equivalent to 39 to 23 per cent.

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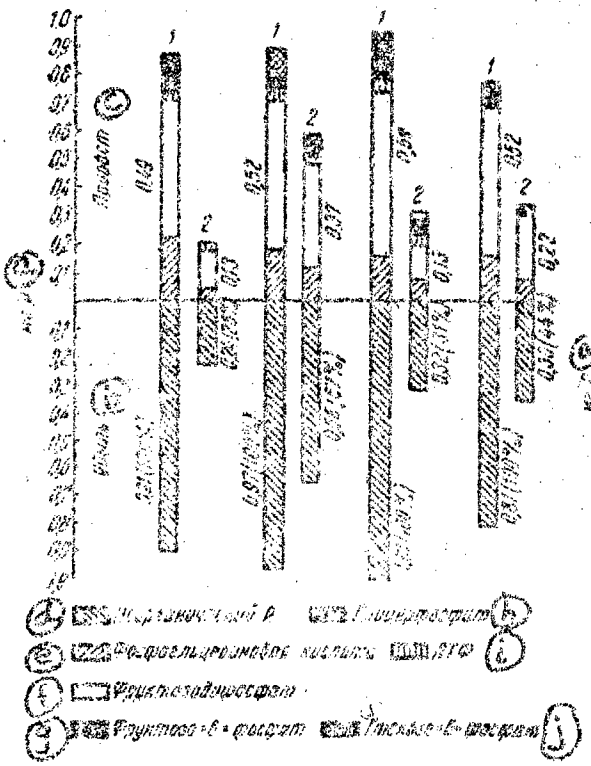
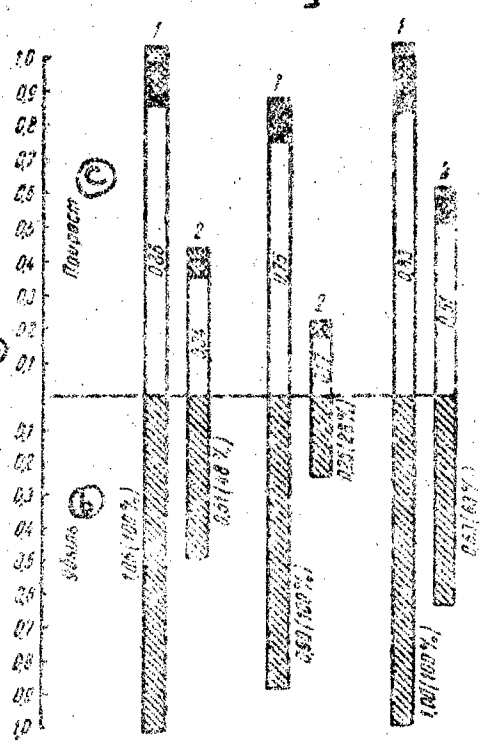
Diag. 1



Diag. 2



Diag. 1. Relationship between phosphorous compounds formed in cerebellar tissue under normal conditions (1) and after irradiation at a dose of 9,000 r (2) (incubation without $CH_2BrCOOH$).
Data represents 4 trials.

Diag. 2. Relationship between phosphorous compounds formed in cerebellar tissue under normal conditions (1) and after irradiation at a dose of 9,000 r (2) (incubation with addition of $CH_2BrCOOH$). Data represents 3 trials.
Dotted columns -- glucose-1-phosphate, remaining symbols are the same as those in diagram 1.

Legend:
(a) mg P
(b) decrease
(c) increase
(d) inorganic P
(e) phospho glyceric acid
(f) fructos diphosphate
(g) fructos-6-phosphate
(h) glyceryl phosphate

(i) ATP (j) glucose-6-phosphate

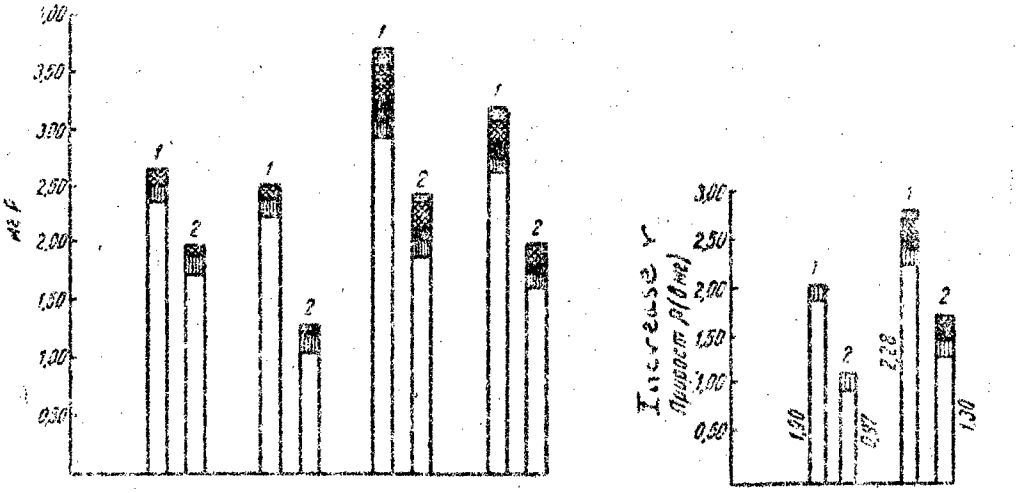
Besides cerebellar tissue, two days after local irridiation of the cerebellum at a dose of 9,000 r, the hepatic tissue was examined. During irridiation of the cerebellum, carbohydrate — phosphorous metabolism is not significantly inhibited in the liver. More marked changes in the distribution of phosphorous fractions were discovered on the second and fifth day of total animal irradiation at a dose of 1,000 r and during cerebral irridiation at a dose of 4,000 r. In determining the phosphorous compounds formed as a result of hepatic tissue carbohydrate — phosphorous metabolism under normal conditions, we found that almost all bound inorganic phosphorous is located in fructos diphosphate fractions, and a very small part of phosphorous ends up in monophosphate esters. X-Ray irridiation, as in local exposure of brain tissue, primarily depresses the formation of fructos diphosphate. In diagrams 3 and 4, the difference in the formation of this compound under normal conditions and after irridiation is clearly demonstrated.

In comparing the distribution of phosphorous compounds in the cerebellum and liver, we must say that if the process of fructos diphosphate formation in cerebellar tissue during local irradiation is established through a determined interval of time while restoring cerebellar functions, then its formation process in the liver decreases daily as a measure of the animal's approaching death. We were able to observe this significant difference only at the peak of radiation sickness.

TABLE 2

Content of cerebellar tissue dry residue before and after irridiation

Material examined	Dry residue in % of damp weight (average amounts)
Cerebellum of control animal	21.9
Cerebellum of experimental animal on the second day after irradiation (severest cerebellar disturbances)	16.9
Cerebellum of experimental animal on the second day after irradiation (moderate cerebellar disturbances)	19.7
Cerebellum of experimental animal on the 14th to 25th day after irradiation (after establishing cerebellar fractions)	21.2



Diag. 3. Relationship between phosphorous compounds formed in hepatic tissue under normal conditions (1) and after irradiation at a dose of 1,000 r (2) data represents four trials.

Diag. 4. Relationship between phosphorous compounds formed in hepatic tissue under normal conditions (1) and after irridiation of the animal's intact head at a dose of 4,000 r (2) data represents two trials.

Symbols are the same in diagrams 3 and 4 as those in diagram 1.

The experimental material for fractionating determination of phosphorous compounds, made possible by clarification of a number of carbohydrate — phosphorous metabolic peculiarities in cerebral and hepatic tissue. Along with the ascertained peculiarities in the distribution of phosphorous fractions formed in these organs, we were able to observe that radioactivity primarily disrupts the formation of fructose diphosphate.

Conclusions

1. During local cerebellar irridiation at a dose of 9,000 r, the oxidative and carbohydrate — phosphorous metabolism is sharply inhibited two days after irridiation of the cerebellar tissue.

2. In irridiated cerebellar tissue the formation of fructose — 1,6 — diphosphate is sharply inhibited. In addition to the fructose diphosphate, but to a significantly lesser degree, the formation of other phosphorous esters is also inhibited. These include phosphoglyceric acid, glyceryl phosphate, glucose-1-phosphate, glucose-6-phosphate, and fructose-6-phosphate.

3. In the case of monobromacetic acid , carbohydrate —

phosphorous metabolism is directed to the formation of fructose-diphosphate, in which case the inhibition of this compound's formation is sharply affected by radioactivity.

4. An insignificant inhibition of carbohydrate — phosphorous metabolism was found in hepatic tissue during local cerebellar irridiation at a dose of 9,000 r.

5. The binding of mineral phosphate in the liver is rather sharply inhibited during irradiation of the intact head at a dose of 4,000 r and also two to five days after total irridiation of the animal at a dose of 1,000 r, as well as at the peak of radiation sickness.

6. Both in the liver and in cerebellar tissue the formation of fructose-1,6-phosphate is primarily deranged.

BIBLIOGRAPHY

1. Golubtsova, A. V., Moiseenko, Ye. V., and Safronova, M. I., Byull. eksper. biol. i med. (Bulletin of Experimental Biology and Medicine), 1956, No 9, p 36.

2. Mytareva, L. V., Med. Radiol. (Medical Radiology), 1956, No 1, p 35.

3. Bekkum, van D. W., Biochim. et biophys. acta, 1955, Vol 16, p 437.

4. Minayev, P. F., Zhurn. obshchey biol. (Journal of General Biology), 1954, No 6, p 401.

5. Minayev, P. F., in the book Sessiya AN SSSR po mirnomu ispol'zovaniyu atomnoy energii. Zasedaniye otdeleniya biol. nauk 1955 g. (Session of the Academy of Sciences USSR on the Peaceful Uses of Atomic Energy. Meeting of the Section on Biological Sciences, 1955), Moscow, 1955, p 51.

6. Sacks, J. J., Biol Chem., 1949, Vol 181, p 655.

7. Skvortsova, R. I., Biokhimiya (Biochemistry), 1953, Vol 18 59[illegible].

8. Meshkova, N. P., and Aleksakhina, N. V., Uspekhi biol. khimii (Advances in Biological Chemistry), 1954, Vol 2, p 27.

ENZYMATIC FORMATION OF HEMOLYSINS IN HEPATIC TISSUE IN RADIATION SICKNESS

-USSR-

[Following is the translation of an article entitled "Fermentativnoye obrazovaniye gemolizinov v tkani pri luchevoy bolezni" (English version above) by V. N. Benevolenskiy in Voprosy Meditsinskoy Khimi (Problems of Medical Chemistry), Vol 6, No 5, Moscow, Sept-Oct 1960, pages 480 to 483.]

Studies of the properties and nature of the hemolytic factor of irradiated animal tissue has shown that it does not in any way differ from normal tissue hemolysins which are formed in the post mortem autolytic disintegration of any tissue. From this fact came the supposition that the appearance of the hemolytic factor in an irradiated organism is tied to the intravital process similar to the autolytic one. In an irradiated organism, the increase in ferment activity which conditions the formation of tissue hemolysins, must be observed. Clarification of this problem is the object of the present work.

Research methods

In accordance with contemporary notions, tissue hemolysins are to be found in all tissues in the form of lipid compounds, unsaturated fatty acids, lysolecithins, and steroids. However, they do not develop hemolytic properties, since they are bound to proteins, cholesterol, calcium and a number of other substances. These ties are disrupted during various phases of intravital and post mortem disintegration which leads to the release of lipid hemolysins and development of tissue hemolytic activity. It is considered that the complex disintegration of lipid hemolysins with their inhibitors, occurs as a result of the action of autolytic ferments of the type protease, lipase and phosphotase. The wide diversity of ferment reactions arising in such cases is far from being studied completely. Therefore the present research was conducted by a method which would at least allow a determination of the total fermenting capability of hepatic water — salt extracts to release tissue hemoly-

sins from inactive tissue preparations, in a hemolytic sense. The principle of this method was proposed by Fonder. The development of method is ours.

Natural water — salt extracts (ferment substrates) were prepared from rat liver which was ground in a glass homogenizer with a four part quantity of physiological solution, and centrifuged at 2500 rpm during a period of 15 minutes.

For use as a mass for the determination of water — salt extract fermenting activity, a system containing fluid of thoroughly boiled hepatic homogenates of normal rats, and washed erythrocytes of the same animals, was utilized.

The liquid mixture was prepared from 25% of hepatic homogenates of normal rats. The homogenate was heated for a period of ten minutes on a boiling water bath and then centrifuged for five minutes at 2500 rpm. The opalescing liquid mixture was slowly poured out and diluted four times with physiological solution. According to Fonder, a certain amount of bound lipid hemolysins will be contained in such a solution in the form of lipoproteins, for the most part. By ferment action, they can be released tested with the aid of erythrocytes.

The erythrocytes used for the test were taken from heparinized blood of normal rats by means of a threefold washing with a ten part amount of physiological solution. A 4% suspension of erythrocytes was used for the experiments.

Every experiment was placed in three systems. The first system consisted of a water — salt extract, and agitated fluid mixture of erythrocytes. The second system consisted of a water — salt extract and of erythrocytes. The third system consisted of an agitated fluid mixture and erythrocytes. The first system was used for the indirect determination of extract fermenting activity. The hemolytic activity of the extract itself was measured in the second system. When there was ferment activity, its true magnitude was equivalent to the difference between the magnitude of hemolysis in the first and second systems. The third system was used as a control for the hemolytic capability of the agitated fluid mixture. When it was present, the experiment was not recorded.

In preparing the first and second systems, the original water — salt extract was diluted by 200 or 400 times with the agitated fluid mixture (for the preparation of the first system), or with physiological solution (for the preparation of the second system).

The formed solutions of all three systems were poured into centrifugal test tubes, each in the amount of 0.5 ml after which to each of these was added 0.1 ml of washed erythrocyte suspension. Then all the test tubes were simultaneously incubated for one hour at 38° and for 48 hours at 5°. Following this, the magnitude of hemolysis was determined with the aid of a photocolorimeter FEK-2, relative to 100% hemolysis in distilled water of an equal quantity of erythrocytes of the same suspension which was used in the given

experiment. Measurement of hemolysis was made with a neutral light filter in cuvettes 1 mm in thickness. Each of the examined solutions was rechecked five times.

During the experiments each of the solutions was measured for pH before and after incubation. The pH measurement was made on a light potentiometer with the aid of a glass electrode. During the period of incubation, the limits of pH change were from 6.6 to 6.4. It was experimentally established that such pH variations in the solutions do not apparently indicate the magnitude of their hemolytic activity.

The experiments were conducted on white male rats weighing from 120 to 160 grams. The experimental rats were irradiated with an X-Ray apparatus RUM-3, under the following conditions: focal distance 40 cm, current 15 ma, tube voltage 180 kw, filter 0.5 mm Cu + 1 mm Al, dose capacity 29 to 30 r/min. The general irradiation dose was equivalent to 1,000 r. At this dosage, the rats usually died 4 to 5 days after exposure. The irradiated animals were killed by means of decapitation after 5 minutes, 1 hour, 1, 2, 3, and 4 days following irradiation.

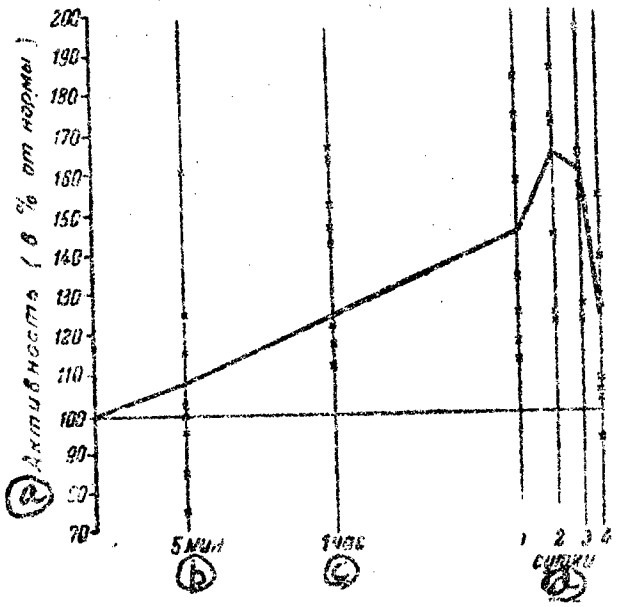
Each experiment was simultaneously placed with extracts from the control (nonirradiated) and experimental (irradiated) rats. Both of these extracts were added to the same supernatant and were tested on identical erythrocytes. A similar organization of periments permitted the examination of the fermenting activity of the tested extracts relative to the control, with maximum accuracy. Taking into account the impossibility of obtaining "standard" erythrocytes, this method may be considered to be most reliable.

In experiments with hepatic autolysis of normal rats, test samples were taken from the same hepatic homogenates after 1, 2, 3 and 4 hours of incubation at 38°. From those test samples, water — salt extracts were prepared and examined by the usual method. The data of the experiments are given according to the results obtained in the examination of extracts from freshly obtained liver. The work was conducted on 112 rats.

Research Results

As a rule, freshly prepared water — salt extracts of liver of the experimental and control rats did not possess hemolytic activity in our concentrations. Exceptions to this rule were exhibited when extracts were used from liver undergoing lengthy autolysis or from rats in the transitory state of radiation sickness. At the time of the addition of these extracts to the supernatant, erythrocytic hemolysis was distinctly expressed. The capability of producing hemolysins from the supernatant was equally possessed by both the extracts of irradiated and nonirradiated rats. However, in the first case the amount of produced hemolysins was, as a rule, greater if the magnitude of observable hemolysis is to be considered. Normally, hymolysis is observed on the addition of control extracts, equivalent to 16 — 22%, in which case the hymolysis was

1½ to 2 times greater than the control when additions of experimental extracts were used. The results of these experiments conducted with extracts of rat liver, where the rats were killed at various stages of the radiation sickness, are represented in the diagram.



Summary ferment activity of water — salt extracts of liver, conditioning the formation of tissue hemolysins during the early stages of radiation sickness of rats (in percentages of normal hepatic extract activity).

Legend:

(a) Activity (in % of the normal)
(b) Minutes
(c) Hours
(d) Days

A curve constructed on the basis of the average data of these experiments indicates, that the ferment activity of hepatic extracts conditioning the appearance of tissue hemolysins during radiation sickness, gradually increases and reaches a maximum on the second to third day. Prior to the animals' death it decreases somewhat.

The activity of hepatic extracts of rats, killed 5 minutes after irradiation, decreased, in a number of cases, in comparison with the observed activity of normal animals. In contrast, in other cases it was increased. Therefore, during this period of determinations no changes were observed.

The results of hepatic extract ferment activity determinations at various stages of autolysis are represented in the table.

(a) Ферментативная активность экстрактов печени, обусловливающая образование тканевых гемолизинов, в процессе автолиза печени нормальных крыс (в процентах к активности свежеприготовленного экстракта)

(b) № опыта	(c) Контроль (свежий экстракт)	(d) Автолизированные экстракты			
		(e) 1 час	2 часа	3 часа	4 часа
1	100	111,1	144,4	122,2	111,1
2	100	170,5	62,3	39,4	1,6
3	100	108,2	66,1	84,8	83,5
4	100	120,3	16,1	10,8	0
5	100	115,6	12,4	0	0
6	100	147	147	117,6	17,6
(f) Среднее . . .	100	128,8	78,1	62,5	35,6

Legend:

(a) Ferment activity of hepatic extracts conditioning the formation of tissue hemolysins during the process of hepatic autolysis of normal rats (in percentages of activity of freshly prepared extract).

(b) Trial number.

(c) Control (fresh extract).

(d) Autolyzing extracts.

(e) Hours.

(f) Average.

From the indicated data, it is apparent that the capability of the autolyzing extracts to release tissue hemolysins varies greatly from experiment to experiment. However, the directional change in the autolytic process remains constant. At first a small increase in activity is observed which soon becomes a decrease. This decrease is lower than the activity level of fresh extract. Therefore, the change characteristics of ferment activity, conditioning the formation of tissue hemolysins, are definitely similar to the dynamics of this change indicator in radiation sickness. The difference primarily lies in the intensity of initial activity increase and subsequent decrease.

In autolysis the former is less significantly expressed than during radiation sickness. On the other hand, the final decrease takes place more sharply.

Discussion of results

The results of our experiments confirm the supposition that the formation of the hemolytic factor in radiation sickness is tied, to a significant degree, to the activity of autolytic ferments which cause the disintegration of tissue hemolysin complexes with their inhibitors. In the process of radiation sickness, it was

found by a number of authors that protease, lipase and phosphotase participate in disintegration marked by an increase in the activity of individual ferments. Similar appearances of certain ferments are observed in studying the disintegration of tissue, such as for example adenosinephosphotase and depolymerase of nucleic acids. These do not directly participate in the formation of the hemolytic factor. Therefore, the increase in ferment activity conditioning the disintegration of tissue similar to that of autolysis exhibits wide distribution in radiation sickness. Normally, such an increase occurs only at a certain period after exposure and becomes strongest at the peak development of radiation sickness. Prior to death the activity of the above mentioned ferments decreases in the majority of cases. An analogous appearance was observed in our experiments.

Similar dynamic changes of summary ferment activity, conditioning the appearance of tissue hemolysins, is observed in the post mortem autolytic process in liver of normal animals. This serves as an additional confirmation of the fact that the disintegration process in the irradiated organism proceeds according to the autolytic mechanism. This process, however, cannot be absolutely confirmed as similar to post mortem autolysis. This is clearly apparent even though some changes discovered by us are found in radiation sickness and autolysis.

Conclusions

1. In liver of irradiated rats with X-Rays (dose 1,000 r) an increase in the hepatic ferment activity occurs one hour after exposure and is observed for a period of 2 to 3 days prior to the animals' death, their summary activity somewhat decreases.

2. A lesser expressed increase in activity of similar ferments occurs in the initial period of post mortem autolysis of normal rat liver. In the later stages of autolysis, the activity of these ferments sharply decreases.

BIBLIOGRAPHY

1. Kudryashov, Yu. B., Dokl. AN SSSR (Reports of the Academy of Sciences USSR), 1956, Vol 109, No 3, p 515.

2. Benevolenskiy, V. N., in the book Pervichnyye protsessy luchevogo porazheniya (Initial Processes of Irradiation Poisoning), Moscow, 1957, p 105.

3. Kazayev, A. P., Ibid., p 132.

4. Zhuravleva, A. I., Luchevoye vozdeystviye na lipidy i lipidnaya model' profilaktycheskogo effekta (Radiation Stimulationof the Lipids and the Limpid Model of Prophylaxis), Avtoref. diss., Moscow, 1959.

5. Gorkin, V. Z., Uspekhi sovr. biol. (Advances in Modern Biology), 1953, Vol 2 (5), p 195.

6. Ponder, E., Red Cell Structure and Its Breakdown, Vienna, 1955.

7. Ponder, E., J. Gen. Physiol., 1951, Vol 34, p 551.

8. Feinstein, R. N., and Ballin, J. C., Proc. Soc. Exper. Physiol., 1953, Vol 83, p 6.

9. Gintsburg, M. B., Pandre, Ye. M., and Binus, N. M., Biokhimiya, 1957, Vol 22, p 467.

10. Tokarskaya, Z. B., Med. radiol. (Medical Radiology), 1959, No 3, p 81.

11. [illegible] G. A. and Natanson, A. O., Eksper. med. (Experimental Medicine), 1936, No 3, p 65.

12. Feinstein, R. N., Radiation Res., 1956, Vol 4, p 217.

13. Prokudina, Ye. A., Tsitologiya (Cytology), 1959, No 1, p 7[illegible]

14. Libinzon, R. Ye., Biokhimiya (Biochemistry), 1959, Vol 2[illegible], p 679.

15. Kuzin, A. M., in the collection Ocherki po radiobiologii (Essays on Radiobiology), Moscow, 1956, p 5.

16. Bacq, Z. M. and Alexander, P., Principes de radiobiology, Paris, 1955.

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ON THE ROLE OF THE LIVER IN THE METABOLISM OF URIC ACID AND ALLANTOIN IN RADIATION SICKNESS

-USSR-

/Following is the translation of an article entitled "O roli pecheni v metabolizme mochevoy kisloty i allantoina pri luchevoy bolezni" (English version above) by T. A. Fedorov and V. P. Fedotov in Voprosy Meditsinskoy Khimii (Problems of Medical Chemistry), Vol 6, No 5, Moscow, Sept-Oct 1960, pages 497-500./

In our previous work, dealing with the effect of - and -rays in lethal doses on the formation of the main end conversion products of nucleic acids — animal uric acid and allantoin, several changes were noticed in the liver activity during the metabolism of these nitrogenous substances.

In the present work, research was conducted on the role of the liver in the metabolism of uric acid and allantoin in dogs poisoned with polonium or irradiated with -rays, during uric acid uptake. Under such conditions, it possible to more specifically characterize the functional state of the liver.

Research Methods

Six dogs (male and female), weighing from 20 to 26 Kg, and sustained on an ordinary diet, were used for the experiment. All the dogs were angiostomized by the London method, modified by Figaiev. Cutaneous cannulae were placed in the portal and hepatic veins. Blood was taken from the portal and hepatic veins, as well as from the femoral artery five to six times at intervals of two to five days. Determinations were made of the experimental dogs' blood serum content of uric acid by the Caraway method, and of allantoin, according to the method of MacPherson and Conway. Since, per unit of time, two-thirds of the hepatic blood enters via the portal vein, and one-third is of arterial origin, the average concentration of the blood components under study was calculated as originating in the indicated ratios.

The uric acid and allantoin plasma content was determined before administration, and five minutes after an intravenous

administration of a uric acid solution, calculated on the basis of 1 mg per 1 Kg of animal weight.

The uric acid solution was prepared in the following manner: 200 mg of uric acid were dissolved in 5 ml of a 0.8% Li_2CO_3 solution at 60°[C], and filtered through a Seitz filter into a flask, containing 20 ml of a 5% glucose solution. One ml of this solution contained 8 mg of uric acid.

After establishing the concentration of uric acid and allantoin the serum of the dogs under normal conditions, and five minutes after the administration of uric acid, two dogs were irradiated with X-rays, and four dogs were poisoned with polonium. The irradiation was conducted by a EGO-2 apparatus with a dose capacity of 460 r/min, and with an irradiation time of 52 seconds. Irradiation was at a dose of 400 r. The animals died on the 9th and 10th day following irradiation. Polonium was administered subcutaneously on a calculated basis of 0.1 microcurie per Kg of body weight. The animals lived for 16 to 20 days, following the administration of polonium.

Research results

The table shows the average data, compiled during examination of healthy dogs before and after uric acid administration, on the uric acid and allantoin content in hepatic affluent and effluent blood. It can be seen that the intravenous administration of uric acid caused an increase in its concentration in the blood of all vessels examined by us. We observed uric acid retention in the liver of healthy dogs, both before and after adminstration of uric acid. An average of 0.18 mg % of uric acid from the blood was taken up by the liver prior to administration, and 0.25 mg % after administration. The intravenous administration of uric acid solution also caused an increase in the blood concentration of allantoin. This is confirmed by the fact that part of the administered uric acid was oxidized to allantoin. We did not observe any hepatic allantoin retention. On the contrary, there was some release by the liver. An average of 0.31 mg % of allantoin was excreted by the liver into the hepatic vein prior to administration, and 0.38 mg % after administration.

The results of these experiments indicate, that a rather large administration of uric acid, under normal conditions, did not cause any noticeable changes in hepatic function, and that only part of the uric acid was oxidized to allantoin in the liver, and this was, for the most part, readily removed from the organism.

Содержание мочевой кислоты и аллантоина в крови, притекающей к печени и оттекающей от нее у собак в норме до нагрузки организма мочевой кислотой и после нагрузки (в миллиграмм-процентах)

Кличка собаки	Количество опытов	Содержание мочевой кислоты						Содержание аллантоина					
		в крови, притекающей к печени		в крови печеночной вены		задержка печенью		в крови, притекающей к печени		в крови печеночной вены		выделение печенью	
		норма	через 5 минут после нагрузки	норма	через 5 минут после нагрузки	норма	через 5 минут после нагрузки	норма	через 5 минут после нагрузки	норма	через 5 минут после нагрузки	норма	через 5 минут после нагрузки
Март[1]	4	0,48	—	0,31	—	—0,17	—	—	—	—	—	—	—
АкБар[1]	4	0,52	0,99	0,40	0,77	—0,12	—0,22	—	—	—	—	—	—
Голубка	8	0,67	0,89	0,48	0,66	—0,19	—0,23	1,42	1,82	1,73	2,34	+0,31	+0,52
Старик	5	0,64	0,82	0,44	0,59	—0,20	—0,25	1,11	1,64	1,43	1,89	+0,32	+0,28
Мартын	5	0,62	0,83	0,42	0,62	—0,20	—0,21	1,72	2,31	2,05	2,70	+0,33	+0,39
Пушок	4	0,68	0,87	0,47	0,55	—0,21	—0,32	1,70	2,06	1,97	2,38	+0,27	+0,32
Среднее значение	30	0,60	0,98	0,42	0,64	—0,18	—0,25	1,49	1,95	1,79	2,33	+0,31	+0,38

[Lecord on following page]

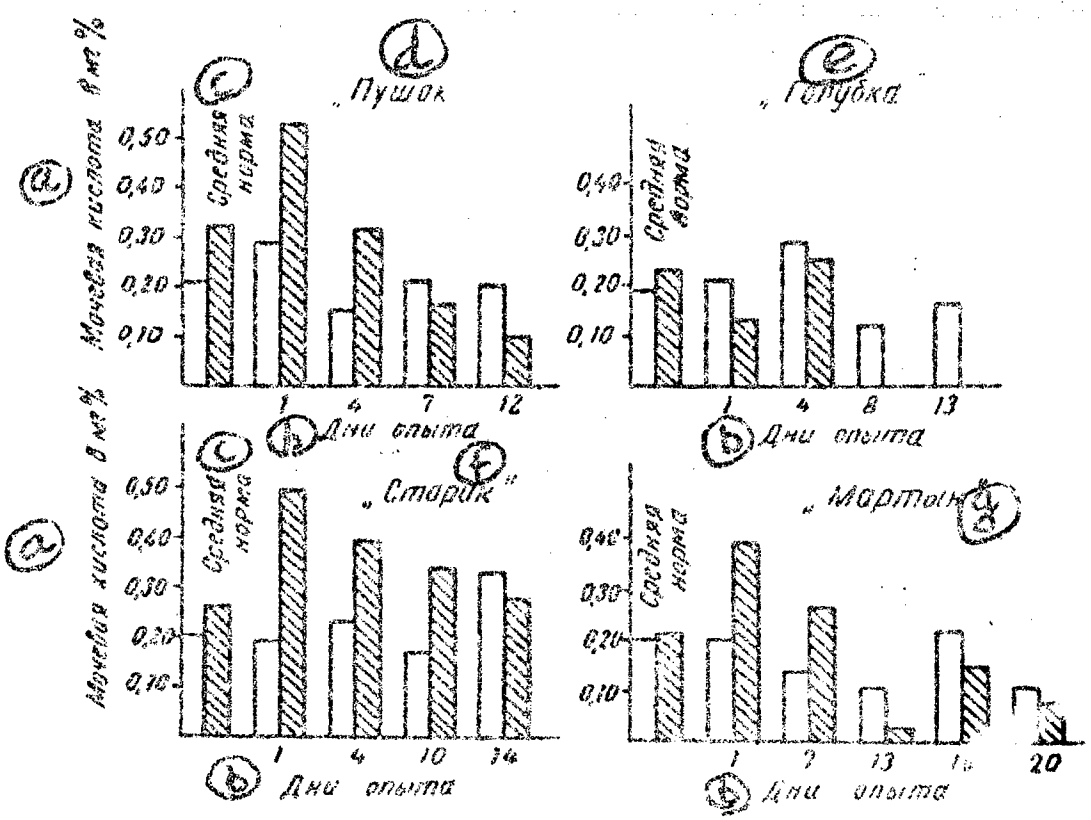
/Following is the legend for the table entitled "Uric acid and allantoin content of hepatic affluent and effluent blood of dogs under normal conditions, before and after administration of uric acid to the organism (in milligrams percent)"./

a) (title as above)
b) Animal name
c) Number of trials
d) Uric acid content
e) Allantoin content
f) Hepatic affluent blood
g) In hepatic venous blood
h) Hepatic retention

j) Hepatic secretion
k) Normal
l) 5 min after administration
m) Mart /See note/
n) Akbar
o) Golubka
p) Starik
q) Martyn
r) Pushok
s) Average value

/Note/ The uric acid in Mart's blood was determined 10 minutes after administration .

Diagram 1 contains data characterizing the changes in hepatic uric acid retention during the development of radiation sickness in each dog poisoned with polonium, before and after the administration of uric acid. The data indicate that twenty four hours after polonium poisoning, hepatic uric acid retention following administration, increased by one and one-half to two times over a period durin which there was hardly any change from the normal, prior to adminstration. This ratio in the hepatic uric acid retention before and after administration, was observed in the dog "Starik" up to the tenth day following polonium poisoning. Uric acid retention by the liver decreased on the fourth day in "Pushok" and on the seventh day in "Martyn", in comparison to the first days, but in both cases post-administrative hepatic uric acid retention was twice as large as before adminstration. In subsequent periods, uric acid administration brought about an opposite effect in all three dogs -- in response to the intravenous administration of uric acid, the liver did not increase, but decreased retention of the former. This paradoxical reaction, on the part of the liver, was observed in "Golubka" during the first days following the administration of polonium.



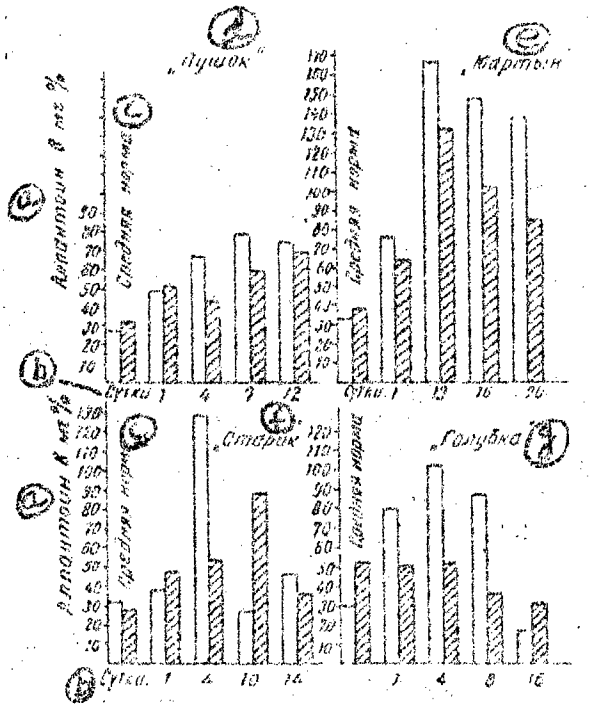
Diag. 1. Changes in the retention of uric acid by the liver of dogs poisoned with polonium, before (clear columns) and after (hatched columns) administration of uric acid.

Legend:

a) Uric acid in mg %
b) days of trial
c) Normal average
d) "Pushok"
e) "Golubka"
f) "Starik"
g) "Martyn"

Changes in the hepatic uric retention in the dogs irradiated with X-rays were of the same character as in those poisoned with polonium. A slight variation, however, consisted of the fact, that the increase in hepatic uric acid retention after administration (after five minutes in "Akbar", and after ten minutes in "Mart"), in comparison to uric acid retention prior to administration, was noticed on the third and fifth days, and the paradoxical hepatic reaction was observed only immediately prior to death.

Diagram 2 contains data on the changes in hepatic allantoin excretion in dogs poisoned with polonium. The allantoin blood content was not determined in animals irradiated with -rays.



Diag. 2. Changes in the excretion of allantoin by the liver of dogs poisoned with polonium, before (clear columns) and after (hatched columns) administration of uric acid.

Legend:

a) Allantoin in mg %
b) days
c) Normal average
d) "Pushok"
e) "Martyn"
f) "Starik"
g) "Golubka"

As has been demonstrated, hepatic excretion of allantoin increased following administration of uric acid to healthy animals (with the exception of "Starik"). After the polonium poisoning of all the experimental animals, it was possible to determine the increase in the hepatic excretion of allantoin before the administration of uric acid. The increase in allantoin excretion was especially high in "Martyn" (from four to five times that of the original norm). The hepatic excretion of allantoin, five minutes after administration of uric acid, in animals poisoned with polonium, also increased in comparison to the normal (with the exception of "Golubka").

The ratio of allantoin excretion, before and after administration of uric acid, changed in the experimental animals during the course of radiation sickness development.

Twenty four hours after polonium poisoning in two of the dogs ("Pushok" and "Starik"), the administration of uric acid caused and increase in hepatic excretion of allantoin, and in two other dogs ("Martyn" and "Golubka"), hepatic excretion of allantoin decreased, following uric acid administration. Subsequently, during the entire period of sickness, and characteristic of the experimental animals' increased excretion of allantoin, the intravenous administration of uric acid caused a decrease in its excretion by the liver. In only two cases ("Starik" -- on the tenth day, and Golubka -- on the sixteenth day), was an opposite effect observed, i.e. the administration of uric acid stimulated the formation of allantoin.

Conclusions

1. In the experiments on dogs poisoned with polonium and irradiated with γ-rays, hepatic retention of uric acid and hepatic excretion of allantoin was increased, i.e. an increased oxidation of uric acid in the initial period of radiation sickness.

2. The oxidative capacity of the liver is noticeably decreased in the terminal period of radiation sickness.

BIBLIOGRAPHY

1. Fedorova, T. A., Fedotov, V. P., and Mkrtumova, N. A., Byull. eksper. biol i med. (Bulletin of Experimental Biology and Medicine), 1959, No 3, p 44.

2. London, E. S., Angiostomie und Organstoffwechsel, Moscow, 1935.

3. Pigalev, I. A., in the book Voprosy patologii i obmena veshchestv. (Questions of Pathology and Metabolism), Moscow, 1950, p 13.

4. Caraway, W. T., Am. J. Clin. Path., 1955, Vol 25, p 840.

5. Young, E. G., MacPherson, C. C., et al, J. Biol. Chem., 1944, Vol 152, p 245.

EFFECT OF CYSTAMINE ON THE LIVER AND SPLEEN CONTENT OF NUCLEIC ACIDS IN IRRADIATED RATS

-USSR-

/Following is the translation of an article entitled "Vliyaniye tsistamina na soderzhaniye nukleinovykh kislot v pecheni i selezenke obluchennykh krys" (English version above) by V. G. Vladimirov in Voprosy Meditsinskoy Khimi (Problems of Medical Chemistry), Vol 6, No 5, Moscow, Sept-Oct 1960, pages 501-505./

At the present time, important disorders of nucleic metabolism have been established in an irradiated organism. These are primarily manifested as disorders in the process of nucleic acid (NA) synthesis and as concentration changes in the tissues.

The favorable effect obtained by the prophylactic administration of certain serum substances in protecting animals against lethal doses of penetrative radiation, has created interest in the study of the effect of these compounds on the content of NA in the various tissues of an irradiated organism.

In research of recent years, by means of observing the spleen and hepatic content of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in irradiated animals, evaluation has been made of the protective activity of a number of substances. The best protective effect was obtained from cystamine (mercaptoethyl amine), which markedly blocked the splenic decrease of NA and normalized its content in the liver.

By means of labeled atoms, there were attempts to explain the effect of these compounds, possessing protective properties, on the speed of NA restoration in radiation sickness. It was shown that in the case of DNA excreted by rat intestine, DNA synthesis was less suppressed in animals to whom cysteine was prophylactically administered, than in those which were unprotected.

In the present work, a study was made of the degree of the protective effect cysteine has on hepatic and splenic NA

in irradiated rats. Cysteine, at the present time, warrants general attention. Since it is a disulfide of cysteamine, it is capable of being restored in vivo to the free cystamine form. In addition to its rather high protective properties, cystamine has a number of other valuable qualities: it is chemically quite stable, and may be taken orally in medical practice.

Research methods

Experiments were conducted on white male rats, weighing from 150 to 230 Gm. The animals were sustained on a normal diet. Radiation sickness was brought about by a single general irradiation with γ-rays of Co^{60} at a dose of 600 r and dose capacity of 7.2 r/min, and a focal distance of 57cm.

The experimental animals were decapitated on the 1st, 2nd, 3rd, 6th, 9th and 14th days, following irradiation. Homogenates of spleen and hepatic tissue were thoroughly washed of lipids and dried in a vacuum exsiccator. The nucleoproteins were extracted with two portions of a 10% NaCl solution at 100° C for two hours. NA determinations were conducted by a method, principally based on their extraction as lanthanum compounds. Alkaline hydrolysis of NA was conducted with a 0.3 N solution of NaOH for 18-20 hours. The DNA was cooled by a 70% solution of $HClO_4$ at pH 1, separated from the RNA fractions by centrifuging, and dissolved by alkalization. The optical density of the separated solutions of DNA and RNA was measured by a spectrophotometer SF-4 at a wave length of 260 mmc. On the basis of the calculated NA content, the following coefficients of optical densities were used: for DNA — 21000 cm^2/Gm; for RNA — 23000 cm^2/Gm. Immediately prior to irradiation, a 1% neutralized solution of this preparation was administered to the animals being studied for the effect of cysteine on hepatic and splenic NA, intra-abdominally, on the basis of 100 mg to 1 Kg of animal wight. The amount of NA in the experimental animals of this series was determined on the 3rd, 9th and 14th day, following irradiation.

Research results

Data in table 1 indicates the NA content in the liver and spleen of non-irradiated rats .

Table 1.
Amount of NA in the spleen and liver of healthy white rats
(in milligrams per 1 Gm of raw tissue weight)

(a) Орган	(b) Количество животных	(c) ДНК (e) пределы колебаний	(c) ДНК (f) среднее арифметическое ($\pm m$)[1]	(d) РНК (e) пределы колебаний	(d) РНК (f) среднее арифметическое ($\pm m$)	(g) РНК/ДНК
(h) Селезенка	25	4,45—10,3	6,47 ($\pm$0,27)	4,0—8,05	5,9 ($\pm$0,2)	0,9
(i) Печень	25	1,32—2,38	1,8 ($\pm$0,06)	4,0—7,4	6,0 ($\pm$0,2)	3,3

[1] $m = \pm\sqrt{\frac{\Sigma a^2}{n(n-1)}}$.

Legend:

a) Organ
b) Number of animals
c) DNA
d) RNA
e) Limits of variation
f) arithmetical mean
g) RNA/DNA
h) Spleen
i) Liver

The splenic content of DNA averaged 6.47 mg, and the RNA content was 5.9 mg per 1 Gm of raw tissue. The average hepatic concentration of DNA was equivalent to 1.8 mg, and the RNA, 6mg per 1 Gm of tissue. These figures coincide with those of the literature. For example, an average of 7.71 mg/Gm of raw tissue of DNA and RNA was found in the spleen by Mandel and his co-workers. Schneider indicates the following amounts for the liver: DNA — 1:94, RNA — 5.2 mg/Gm of tissue. There was a somewhat larger quantity of RNA obtained in other works. However, it should be remembered, that the RNA content largely depends on the diet, age of the animals and other factors.

A reduction in the spleen content of DNA was noticed 24 hours after irradiation of the animals. By the third day, the amount of DNA was at its lowest level, an average of 3.06 mg/Gm of tissue weight. This was approximately 47% of the non-irradiated rat level (see table 2).

There was a subsequent gradual increase in the DNA content, but complete normalization did not even occur by the fourteenth day.

Table 2.
Amount of NA in the spleen of rats irradiated at a dose of 600 r (in milligrams per 1 Gm of raw tissue weight)

(a) Сутки после облучения	(b) Количество животных	(c) ДНК		(d) РНК		(e) РНК/ДНК
		(f) пределы колебаний	(g) среднее арифметическое (±m)	(f) пределы колебаний	(g) среднее арифметическое (±m)	
1-е	12	1,57—7,05	4,79 (±0,48)	2,76—6,35	4,77 (±0,36)	
2-е	14	1,97—5,0	3,77 (±0,26)	3,36—8,03	4,54 (±0	1,2
3-и	18	1,74—3,86	3,06 (±0,24)	2,6—7,05	3,59 (±0,26)	1,3
6-е	12	1,71—5,22	3,89 (±0,26)	3,52—11,1	6,64 (±0,6)	7
9-е	8	2,68—7,48	5,05 (±0,42)	3,46—9,0	6,64 (±0,64)	1,3
14-е	13	3,24—6,55	5,13 (±0,34)	4,0—8,76	7,24 (±0,4)	1,4

Legend:
a) Days after irradiation
b) Number of animals
c) DNA
d) RNA
e) RNA/DNA
f) Limits of variation
g) arithmetical mean

The amount of RNA in the spleen also decreased during the first days following irradiation, however; not as sharply as in the case of DNA. By the sixth day, the splenic amount of RNA was restored, and by the 14th day, its level seven exceeded, by approximately 13%, the average level of healthy dogs.

The effect of irradiation on the hepatic concentration of DNA was reduced. However, the reduction was less distinct than in the spleen, but the normalization of DNA in the liver was more pronounced (table 3.). The changes in hepatic RNA during irradiation differ somewhat from those observed in the spleen. From the second day, in the case of the liver, there was a RNA increase, which, by the ninth day, reached a maximum, and exceeded the normal by more than 60%.

Table 3.
Amount of NA in the liver of rats irradiated at a dose of 600 r (in milligrams per 1 Gm of raw tissue weight)

(a) Сутки после облучения	(b) Количество животных	(c) ДНК (f) пределы колебаний	(c) ДНК (g) среднее арифметическое ($\pm m$)	(d) РНК (f) пределы колебаний	(d) РНК (g) среднее арифметическое ($\pm m$)	
1-е	12	0,95—1,93	1,6 (±0,12)	4,05—6,95	5,86 (±0,25)	3
2-е	14	0,83—2,4	1,39 (±0,13)	4,28—8,26	6,74 (±0,25)	4
3-и	18	0,77—2,0	1,22 (±0,07)	4,44—7,73	6,13 (±0,3)	5
6-е	12	1,1—2,48	1,55 (±0,15)	4,9—18,5	9,03 (±1,12)	5
9-е	9	1,17—3,06	2,14 (±0,19)	6,3—11,8	9,74 (±0,72)	4
14-е	13	1,11—2,92	2,03 (±0,18)	5,27—9,95	7,66 (±0,39)	3

Legend: As in table 2.

Having obtained the NA content change in the liver and spleen during the course of radiation sickness, we attempted to prevent their reduction by means of the prophylactic administration of cystamine. The protective activity of cystamine was determined on the 3rd, 9th, and 14th days following irradiation of the animals. In a like manner, it was determined that the greatest variation changes of NA occurred on the 3rd day, both in the liver and spleen, and that on the ninth day, there were the distinct characteristic processes of normalization of NA content.

Finally, it was of interest to know in what manner could the prophylactic administration of cystamine predict the NA content in the organs under study, at future dates. The data of this series of experiments are presented in table 4. It is interesting to note, that a similar biphasic increase in the DNA content was observed in radiosensitive organs, when using cystamine solutions as protective.

Table 4.
Amount of NA in the spleen and liver of irradiated rats to whom a prophylactic solution of cystamine was administered (in milligrams per 1 Gm of raw tissue weight)

(a) Сутки после облучения	(b) Количество животных	(c) ДНК: (f) пределы колебаний	(g) среднее арифметическое (±m)	(h) процент к количеству у облученных	(d) РНК: (f) пределы колебаний	(g) среднее арифметическое (±m)	(h) процент к количеству у облученных	(e) РНК/ДНК
			(i) Селезенка					
3-и	10	4,05—6,53	5,35 (±0,18)	175	3,32—7,05	4,9 (±0,42)	122	0,9
9-е	10	3,97—6,65	1,92 (±0,27)	97	6,42—9,9	7,15 (±0,53)	108	1,4
14-е	12	2,7—12,90	7,20 (±1,16)	140	5,44—9,0	7,62 (±0,32)	105	1
			(j) Печень					
3-и	10	1,33—2,88	1,81 (±0,15)	148	5,06—8,22	6,4 (±0,31)	104	3,5
9-е	10	1,28—2,13	1,75 (±0,11)	82	4,0—10,8	7,33 (±0,67)	75	4,2
14-е	11	1,11—2,96	2,03 (±0,24)	100	5,23—9,32	6,9 (±0,45)	91	3,4

Legend: a) through g) as in table 2.
h) percentage of the number of irradiated
i) Liver j) Spleen

As can be seen from table 4, the prophylactic administration of cystamine led to the prevention of DNA reduction in the early periods following exposure to penetrative radiation. By the third day, following irradiation, the splenic content of DNA was 75% higher, and the hepatic content 48% higher, than in the organs of irradiated animals to whom cystamine was not administered. There was some increase in the amount of RNA in the spleen of rats protected with cystamine.

By the ninth day, no distinctive effect of cystamine on the DNA content of organs under study, was observed. By this time, the amount of hepatic RNA in the protected animals was somewhat less (by 25%) than in the control. By the 14th day, there was observed a small new increase of the DNA level in the spleen of the protected animals. It is considered that the increase in the DNA level, observed in irradiated animals by the end of the second week, is conditioned by intensive tissue regeneration,

originating in the protective cells.

The mechanism of the protective action of cystamine, as well as cysteine, is not yet sufficiently clear. Ferments, containing SH- groups have been found to be most sensitive to ionizing irradiation. Under the stimulation of penetrating radiation, in several cases, there was oxidation of the SH- group to disulfide, in other cases, several ferments were converted to restorative processes. It may be assumed that the positive effect of administering such compounds contained in the plasma, in radiation sickness, is tied to the ability of these substances to maintain the intra-cellular thio ferments which take part in NA synthesis. It must also be considered that compounds of a similar type can influence the level of oxidative restorative processes, or interact with the oxidizing radicals formed by irradiation.

However, whatever the disruptive mechanism of nucleic metabolism may be, it is evident that such disorders play an important role in the development of radiation sickness. Cystamin prevents, to a significant degree, NA metabolic disorders, arising from exposure to penetrating radiation. The study of the action of protective substances on metabolism helps to clarify the affection process mechanism, and facilitates the search for new and more effective means.

Conclusions

1. General irradiation with γ rays of Co^{60} at a dose of 600 r, causes a NA content reduction in the spleen of white rats, which is especially pronounced by the third day.

Following irradiation, the amount of DNA in the liver decreased to a lesser degree than in the spleen, but normalization takes place significantly faster.

2. A prophylactic administration of a 1% solution of cystamine, on the basis of 100 mg per 1 Kg of animal weight, significantly prevents the reduction of the NA content in the spleen, and the DNA content in the liver, in the first days following irradiation. Its effect was not distinct at later periods.

BIBLIOGRAPHY

1. Milic, D. and Nosek, J., Cas. lek. ces., 1958, Vol 97, p 208.

2. Gros, C. and Mandel, P., Comp rend. Sci., 1953, Vol 236, p 2010.

3. Hagen, U., Ernst, H., and Langendorff, H., Strahlentherapie, 1958, No 107, p 426.

4. Mole, P. H. and Temple, D. M., Nature, 1957, Vol 180, p 1278.

5. Henke, H., Manss, H. and Schubert, G., Strahlentherapie, 1958, No 106, p 253.

6. Ivanova, T. N. and Pravdina, N. I., Dokl. ANSSSR (Reports of the Academy of Sciences USSR), 1954, Vol 95, No 4, p 845.

7 Nyurnberger, Deh. I., in the book, Metody tsitologicheskogo analiza (Methods of Cytological Analyses), Moscow, 1957, p 179.

8. Jacob, K., Mandel, L., and Mandel, P., Experientia, 1951 Vol 7, p 269.

9. Schneider, W. C., Hogeboom, G. H., and Shelton, E., et al, Cancer Res., 1953, Vol 13, p 286.

10. Thomson, R. Y., Reagy, F. C., Hutchison, W. C., et al, Biochem. J., 1953, Vol 53, p 460.

11. Scherer, E., Klin. Wschr., 1936, No 34, p 95.

12. Isachenko, V. B., Vestn. rentgenol. i radiol. (News of Roentgenology and Radiology), 1956, No 9, p 5.

13. Romantsev, Ye. F. and Suvich, A. V., Khimicheskaya zashchita ot ioniziryushchey radiatsii (Chemical Defense Against Ionizing Radiation), Moscow, 1958.

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